EXHIBIT H

3 Scott-Ham M, Burton FC. Toxicological findings in cases of alleged drug-facilitated sexual assault in the United Kingdom over a 3-year period. *J Clin Forensic Med* 2005; **12:** 175–86.
4 Payne-James J, Rogers D. Drug-facilitated sexual assault, 'ladettes' and alcohol. *J R Soc Med* 2002; **95:** 326–27.

Non-invasive prenatal diagnosis of Down's syndrome

One of the most remarkable aspects of the report by Ravinder Dhallan and colleagues (Feb 10, p 474)[1] is the substantial increase in the proportion of fetal DNA in relation to maternal DNA that is achieved by simply adding formaldehyde to the tubes after blood has been drawn from the pregnant woman. The protocol seems to be the same as published before, but which could not be reproduced by two interim studies.[2,3] It is curious that there is no mentioning of this controversy in the *Lancet* paper.

Before jumping to conclusions that we have now entered a new era in non-invasive prenatal diagnosis by way of the formaldehyde approach in combination with multiplex single-nucleotide polymorphism analysis, we will require a more detailed protocol and a logically explained mode of action for the formaldehyde treatment. Further confirmation of its efficacy for enrichment of fetal DNA in maternal plasma is needed before it can be used for non-invasive prenatal diagnosis of common chromosome disorders such as Down's syndrome in routine clinical practice.

It should be noted that there has recently been substantial progress in the development of biomarkers for non-invasive prenatal diagnosis of common numerical chromosomal abnormalities.[4,5] Risk-free prenatal diagnosis of Down's syndrome could indeed be a viable clinical reality within the next 6–12 months or so.

MH is director of Simeg Ltd and has filed patents on non-invasive prenatal diagnosis technology.

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1 Dhallan R, Guo X, Emche S, et al. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* 2007; **369:** 474–81.
2 Chinnapapagari SK, Holzgreve W, Lapaire O, Zimmermann B, Hahn S. Treatment of maternal blood samples with formaldehyde does not alter the proportion of circulatory fetal nucleic acids (DNA and mRNA) in maternal plasma. *Clin Chem* 2005; **51:** 652–55.
3 Chung GT, Chiu RW, Chan KC, Lau TK, Leung TN, Lo YM. Lack of dramatic enrichment of fetal DNA in maternal plasma by formaldehyde treatment. *Clin Chem* 2005; **51:** 655–58.
4 Lo YM, Tsui NB, Chiu RW, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nat Med* 2007; **13:** 218–23.
5 RW Old, F Crea, W Puszyk, M Hultén. Candidate epigenetic biomarkers for non-invasive prenatal diagnosis of Down syndrome. *Reprod Biomed Online* (in press).

We are intrigued by the study by Ravinder Dhallan and colleagues,[1] having a long-standing interest in prenatal diagnosis ourselves.[2]

First and foremost, the controversy surrounding fetal DNA enrichment by formaldehyde is not mentioned.[3,4]

Second, the test involves the analysis of 549 and 570 single-nucleotide polymorphisms (SNPs) on the target chromosomes in the maternal and paternal DNA samples and three maternal plasma replicates, amounting to an impressive total of 5595 sequencing gel readings per pregnancy. The variability in the SNP ratios among the replicate analyses, and thus the robustness of the test, were not reported. Would cases be scored similarly as trisomy 21 or normal if the chromosomal ratio comparisons were done separately for each replicate?

Third, if the expected SNP heterozygosity rates were disclosed, readers could independently determine the expected mean number of fetal-specific SNPs. We were alarmed that none of the fetal genotypes were confirmed by use of fetal tissues. From maternal plasma alone, the reported number of fetal-specific SNPs could represent only a subset of all paternally inherited unique SNPs. In fact, we saw from Dhallan and colleagues' data a correlation between the total number of detectable fetal SNPs and the proportion of fetal DNA (Spearman correlation, r=0·3, p=0·02). This observation suggests that the SNP number is not solely determined by the fetal genotype but confounded by the amount of fetal DNA.

We were also puzzled that the ratios were derived from a variable number of SNPs per case and per chromosome, implying that the test would have variable precision and different statistical power from case to case.

We have filed patent applications on aspects of prenatal diagnosis. Some of these intellectual properties have been licensed to Sequenom, Core Healthcare, and Institut Jacques Boy, with licensing income.

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1 Dhallan R, Guo X, Emche S, et al. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* 2007; **369:** 474–81.
2 Lo YMD, Chiu RWK. Prenatal diagnosis: progress through plasma nucleic acids. *Nat Rev Genet* 2007; **8:** 71–77.
3 Chinnapapagari SK, Holzgreve W, Lapaire O, Zimmermann B, Hahn S. Treatment of maternal blood samples with formaldehyde does not alter the proportion of circulatory fetal nucleic acids (DNA and mRNA) in maternal plasma. *Clin Chem* 2005; **51:** 652–55.
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Ravinder Dhallan and colleagues[1] allude to the possible non-invasive detection of fetal aneuploidies by the quantitative analysis of paternally inherited single-nucleotide polymorphisms (SNPs) in cell-free fetal DNA. Although very high degrees of specificity were claimed, only two out of three cases with trisomy 21 were detected. Therefore this approach is no improvement over current screening strategies that use combinations of ultrasound and serum analytes. It is also inferior to that attained in a report in which cell-free fetal mRNA was used.[2]

For this approach to be accepted in clinical practice, it will need to be

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verified independently, preferably in large multicentre studies. There are, however, several issues which will make an independent assessment difficult. These include the selection of candidate SNP loci, which is described in a patent not readily available to the research community. Additionally, how a quantitative procedure relying on numerous steps (preamplification, PCR, biotin-mediated capture, fluorescent labelling, electrophoresis, and digital imaging) can permit results involving 4 decimal places is unclear.

Finally, this study relies on a former report wherein the authors suggested that the use of formaldehyde would increase the proportion of cell-free fetal DNA in maternal blood samples[3]—a feature which could not be reproduced in several independent studies.[4,5] Hence this report in its current form could be too preliminary.

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1 Dhallan R, Guo X, Emche S, et al. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* 2007; **369:** 474–81.
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Authors' reply

In researching methods to increase the proportion of fetal DNA recovered from maternal blood, and developing non-invasive methods for prenatal diagnosis, the main objective should be to contribute practical solutions to real-world problems.

Blood samples must be routinely transported from laboratories or physicians' offices to a testing facility and then processed, and there is great potential for maternal cell lysis because of mechanical shearing and degradation resulting from shipping, chemical processing, and centrifugation. Our formaldehyde method was developed to address this real-world problem. Addition of formaldehyde to maternal blood samples significantly increases the proportion of fetal DNA recovered from the plasma.[1,2] Benachi and colleagues[3] confirmed our results, reporting a range of 5·6–96% fetal DNA and a mean of 36·8% fetal DNA recovered from maternal plasma. We are perplexed by the apparent inability of others to practise this technique.

Fetal genotypes were confirmed by genotyping parental genomic DNA. Paternal genotyping was used for reference purposes to reduce the number of single-nucleotide polymorphisms (SNPs) analysed in the plasma, and is not required for quantitative analysis of fetal DNA. We compared our results with amniocentesis or newborn reports from the clinical sites. Our aim is to develop a completely non-invasive test not reliant on analysis of invasively obtained samples. Presentation of methods as "non-invasive" when they clearly rely on analysis of invasively obtained tissues is misleading.[4,5]

Sinuhe Hahn and colleagues state that our approach "is inferior to that attained in a report in which cell-free fetal mRNA was used." If a comparison is to be made with other methods, it should at least be done accurately. We sequentially analysed 60 samples, none of which were excluded from our statistical analysis.[2] In the paper cited in comparison as superior, Lo and colleagues collected and analysed 119 samples; however, 52 (44%) did not have a heterozygous phenotype at the locus of interest, and were removed from the dataset before statistical analysis. Lo and colleagues then reported successful identification of 55 of 57 normal samples and nine of ten trisomy 21 samples and estimated specificity and sensitivity based on 67 rather than the 119 samples collected, artificially improving their results. They fail to acknowledge that their methods were only diagnostic for 56% of the samples they collected.

As discussed in our paper, it is evident that the number of SNPs visible in the plasma often, although not consistently, relates to the proportion of fetal DNA in the sample. An additional practical benefit of this method is its ability to be done in the first trimester, allowing for analysis of a follow-up sample should a low number of SNPs be identified in the initial sample. Furthermore, unlike methods based on quantification of one or few SNPs on one or few chromosomes,[4,5] the approach we describe is unlimited in terms of the number of reference chromosomes and number of SNPs that could be added to the analysis.

RD is the founder, chief executive officer, and chairman of the board of directors of Ravgen Inc, and a stockholder of that company. XG and SE are employed by, and have options to purchase stock in, Ravgen. MD is an unpaid member of Ravgen's advisory board, and has options to purchase stock. PB is a member of the board of directors of Ravgen and has options to purchase stock. Ravgen Inc has been issued patents and has multiple patent applications pending for the methods described in this letter.

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